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Note

Simple determination of trichloroacetic acid in urine using head space gas chromatography: a suitable method for monitoring exposure to trichloroethylene

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Trichloroethylene is a relatively frequent industrial toxic substance. It is a well known fact [1] that 60—75% of trichloroethylene absorbed by the exposed organism is mainly metabolized to trichloroacetic acid (TCA), and to trichloroethanol as well. Perchloroethylene is also metabolized to TCA to a limited extent [2].

TCA has a longer half-time of excretion (100 h) compared with trichloroethanol (12 h) [3]. Hence the concentration of TCA in the urine characterizes a week's exposure, while that of trichloroethanol characterizes the exposure of the previous working shift [4]. In evaluating the permanent but mostly variable exposure to trichloroethylene, the determination of TCA in the urine is more significant if compared with that of trichloroethanol. Moreover, during exposure to perchloroethylene, trichloroethanol is not present in the urine at all.

Hence we paid particular attention to developing a method suitable for the determination of TCA in the urine in our current screening of workers' exposure to trichloroethylene or perchloroethylene. In the literature can be found many methods. Photometric determination based on Fujiwara's reaction is the most frequently applied method for the determination of TCA, even in outdoor practice [1, 5]. Another method increasingly being used is that of gas chromatography. For this purpose TCA is changed mostly into lower polarity esters, determined either after extraction into organic solvent [6], or directly in the aqueous phase using head space analysis [7, 8] or electron-capture detection. The simple method with flame ionization detection [9] based on direct injection of urine into the gas chromatograph is very interest-

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ing. Thermal decarboxylation of TCA occurs and results in the formation of chloroform, the peak of which is recorded. This otherwise very rapid procedure is disadvantageous since the column becomes contaminated with urinary components of higher boiling point.

In order to remove the biggest disadvantages of the above methods, i.e. complicated procedure, necessity of electron-capture detection, rapid pollution of the column, etc., we developed a method of our own. In this report we demonstrate the possibility of simple determination of TCA in the urine using gas chromatography. By heating a sample of urine in a closed flask, decarboxylation of TCA to chloroform is attained, immediately determined by head space analysis and evaluated by comparison with an internal standard.

EXPERIMENTAL

Materials

Aqueous solution of TCA p.a., 1000 mg/l, was from Cambrian Chemicals, Beddington, U.K.; *n*-butanol p.a. (Lachema, Brno, Czechoslovakia) was redistilled before use.

Sample bottles were flasks (25.0 ml) with screw cap perforated in the centre, with a 2 mm thick silicone rubber lining covered with aluminium foil.

Procedure

A 5-ml volume of urine was pipetted into a 25-ml flask with 10 μl of *n*-butanol. The flask was closed and placed in a thermostatically controlled water bath. After 75 min at 90°C decarboxylation of TCA to chloroform was attained and the flask was slowly transferred to a water bath maintained at 45°C. After 20 min, 1 ml of gas phase was taken with a heated syringe within 10—15 sec and injected into the gas chromatograph.

Gas chromatography

A gas chromatograph Chrom 42 (Laboratorní Přístroje, Prague, Czechoslovakia) with a flame ionization detector was used. A glass column (2.5 m × 3 mm I.D.) was packed with 10% Carbowax 20 M (Applied Science Labs., State College, PA, U.S.A.) on Chromosorb W AW, 100—120 mesh (Supelco, Bellefonte, PA, U.S.A.). The injector block was maintained at 140°C and the column at 130°C. Flow-rates were 500 ml/min for the air, 30 ml/min for the hydrogen and 70 ml/min for the carrier gas (nitrogen). The sensitivity used was 1:200.

RESULTS

Fig. 1 shows a typical chromatogram obtained from the analysis of a urine sample from a person exposed to trichloroethylene. A small peak with a retention time of about 1 min had no connection with the concentration of TCA and was therefore not studied further.

Calibration was done both by analysing standard aqueous solutions of TCA in the range 0—250 mg/l, and by adding increasing amounts of standard to urine for determining any interference from other substances in the urine. The

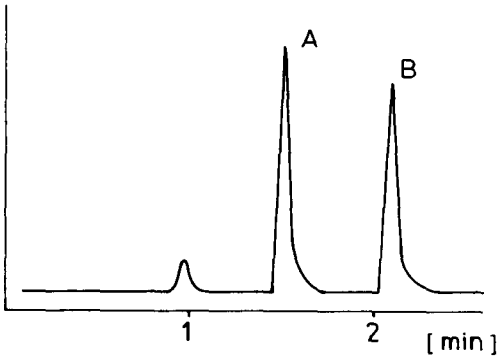

Fig. 1. Typical chromatogram obtained by analysing the urine of a worker exposed to trichloroethylene (urine concentration of TCA 48 mg/l). Peaks: A = chloroform: B = *n*-butanol (internal standard).

TABLE I

PRECISION AND RELATIVE RECOVERY OF URINARY TCA DETERMINATION

$n = 10$.

TCA conc. + standard (mg/l)	Mean (mg/l)	S.D. (mg/l)	C.V. (%)	Relative recovery (%)
9.0 + 0	9.0	0.6	8.5	
9.0 + 125.0	139.5	5.8	7.0	104.3
9.0 + 250.0	246.3	13.0	9.1	95.1

course of the calibration curve was linear in both cases in the indicated range, the lines having the same slope. Thus interference by other substances in the urine was excluded.

Precision and relative recovery of the method were verified by ten-fold repetition of the determination, both in the urine of an exposed worker and in the same urine with increasing additions of standard TCA. For the results see Table I.

The sensitivity of the method was about 2 mg/l.

To control the results, urine samples of persons from three places of work who were exposed to trichloroethylene were determined using both the photometric method and head space analysis. The results are compared in Table II.

DISCUSSION

The time required for the decarboxylation of TCA to chloroform was verified experimentally. During the first 45 min the peak of chloroform is increasing. A maximum is reached within about 60 min and is not changed by further heating. Optimum conditions were taken as 75 min heating at 90°C approximately. The temperature of the bath, 90°C, was chosen on purpose. At this temperature there is no boiling so that a water thermostat can be used, although simply submerging the flask in a vessel of boiling water could obviously also be done. At lower temperatures the period of heating should be prolonged.

TABLE II

COMPARISON OF RESULTS OBTAINED USING THE PHOTOMETRIC METHOD AND HEAD SPACE ANALYSIS

Work place	Subject examined		Concentration of TCA (mg/l)		Comment
	Sex	Age (years)	Photometry	Head space analysis	
Dry cleaning	M	31	23	25	Exposure at admissible level
	F	28	48	47	
	F	28	53	51.5	
	F	40	39	41	
		Mean:	40.75	41.1	
Production of musical instruments (cleaning of brass parts)	M	25	68	72.5	Surpassing the admissible level [10]
	M	32	123	120.5	
	M	44	105.5	108	
	F	27	55	55.5	
	F	28	81	80	
	F	45	115	120	
	F	48	70	76	
		Mean:	88.1	90.3	
Electronics production (cleaning of small parts)	F	28	6	6.5	Low exposure
	F	36	15	14	
	F	50	8.5	8	
	F	33	9	9	
	M	57	12.5	14	
		Mean:	10.2	10.3	

The reason for using another water bath at 45°C is one more of practice than of principle, since it is easier to take a sample of the gas phase from a flask at 45°C than at 90°C. Also this makes the method more reproducible since 45°C is nearer the temperature of the laboratory and thus there is not such a great temperature difference between the syringe and the sample.

The high temperature necessary for the decarboxylation of TCA to chloroform requires paying great attention to the perfect packing of the flasks for head space analysis. We would advise, for instance, to use flasks with ground glass joints covered with a thin layer of silicone oil. The rubber packing should be lined with aluminium foil to prevent undesirable absorption of chloroform into the rubber. In order to exclude the possible production of chloroform from trichloroethanol, the procedure was applied to aqueous solutions of 250 mg/l trichloroethanol (Koch Light, Colnbrook, U.K.). In these conditions no peak for chloroform was recorded.

In comparing the results of the suggested head space analysis (see Table II) with those obtained by the photometric method [1, 5], it was found that in three working places with various exposures to trichloroethylene the methods were equivalent. Thus the long-term practice of applying the photometric

method confirmed the applicability of the suggested procedure for monitoring the exposure of workers. There is, however, a certain disadvantage of the suggested method compared with that of Buchet et al. [9], namely a slightly longer time needed for the analysis of one sample. This is, however, considerably shortened in series analysis. Moreover, if the time necessary for cleaning the column contaminated with volatile urinary constituents is also taken into consideration, the utility of the suggested method in analysing a larger amount of samples is quite evident.

The studies of Breimer et al. [7], Ogata and Saeki [6] and Monster and Boersma [8] report the simultaneous determination of trichloroethanol as well, but in determining TCA alone the procedure is much simpler, less elaborate, and does not require electron-capture detection since flame ionization detection and a relatively low sensitivity are sufficient.

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